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GERMICIDAL ACTION AND SPECIFIC BACTERIOTROPISM OF QUININ AND UREA HYDROCHLORID, ETHYLHYDROCUPREIN, AND OTHER CINCHONA DERIVATIVES ON PNEUMOCOCCI IN VITRO*

STUDIES IN PNEUMONIA, I

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It has been long and generally known that quinin possesses certain antiseptic and bactericidal properties, and there is a long-standing tradition, endorsed by such clinicians of an elder day as DaCosta[1] and Bartholow,[2] that quinin given sufficiently early and in sufficiently large dose, will arrest an attack of pneumonia. Many physicians have advocated — as others with equal earnestness have opposed — the use of quinin salts as a routine treatment in this malady.† Among the former, Juergensen,[3] Schultz,[4] Aufrecht and Petzold,[5] Henry,[6] Gibson,[7] and Galbraith[8] may especially be cited. One of us (S. S. C.)[9] has for many years — since 1904 systematically, as part of a definite plan for the

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† Since this clinical testimony is at present largely ignored or forgotten, a few illustrative quotations from teachers of international repute, as well as from a country practitioner of large experience, are worth making note of.

"Administered at the critical moment," says Bartholow (1876), "a commencing fibrinous pneumonia . . . may be suppressed by a full dose (20 to 40 grains). Its power in this respect is much increased by combination with morphia. If the time has passed for the use of quinia in this way, it is employed with advantage . . . in . . . small doses (2 to 4 grains) . . . given frequently."

One of us (S. S.-C.) repeatedly heard DaCosta state in the course of that master's lectures on the practice of medicine (about 1880) that full doses of quinin administered shortly after the initial chill, will often arrest the development of acute lobar pneumonia. "It is difficult to prove this fact," DaCosta

* Received for publication November 27, 1916.

[1] From a student's notes of lectures (1880 to 1883).

[2] Materia Medica and Therapeutics, 1878, p. 141.

[3] Von Ziemssen's Cyclopedia of the Practice of Medicine (Eng. translation), 1875, p. 165.

[4] Jour. Am. Med. Assn., 1886, 7, p. 119.

[5] Deutsch. Arch. f. klin. Med., 1901, 70, p. 373.

[6] Tr. Assn. Am. Phys., 1911, 26, p. 175.

[7] Glasgow Med. Jour., 1911, 75, p. 321.

[8] Jour. Am. Med. Assn., 1904, 43, pp. 108, 608.

[9] Tr. Assn. Am. Phys., 1911, 26, p. 169. Am. Jour. Med. Sc., 1912, 143, p. 40.

treatment of pneumonia — employed large doses of the double hydrochlorid of quinin and urea by subcutaneous and intramuscular injection and, more recently, by intravenous injection; and has made a number of reports of its apparently favorable influence.[10]

The research of which the present communication reports but a single preliminary phase, is the long delayed fruition of his hope to put to experimental test certain opinions formed and expressed as the result of the therapeutic experience cited. Altho the friend and patient whose generosity has made it possible to begin the work declines to have his name attached to it, that generous assistance may nevertheless be acknowledged thus impersonally.

The validity of the clinical observations cited being assumed, we

would go on to say, "because one has no means of showing that what is absent was on the way. Yet it is true. The fact that pneumonia sometimes aborts spontaneously, or runs a very short course, does not negative this view. The real difficulty is that one rarely sees his patient soon enough."

Perhaps the most outspoken of modern champions of the use of quinin in pneumonia is Juergensen (1871), who writes:

"For children I use a grain and a half (0.1 Gm.) for every year up to five years of age, and after that period from seven to fifteen grains (0.5 to 1 Gm) according to circumstances. These quantities may be exceeded without doing any harm. When the fever is intense, seventy-seven grains (5 Gm.) may be given to a strong adult, and fifteen grains (1 Gm.) to a child under one year, always in one dose. I have repeatedly used both these amounts. I have acquired my experience by gradually increasing my doses, and I have never seen any harm done; in fact it is my firm belief that even these are not the extreme limits as to quantity. . . . I know that many will be alarmed at such large doses. My teacher is experience. Only fools resist facts. . . . The heart is the guide. . . . Whoever carefully examines the pulse walks safely; whoever fails to do it, easily stumbles. I have never noticed any harm done by quinin. The pulse falls at the same time with the temperature, but remains full and strong, or is improved in character if it had previously been weak. The discomfort on the part of the patient is generally not much greater than from small doses, and disappears rapidly."

O. T. Schultz of Mt. Vernon, Ind., "a small country town in the confluence of the Ohio and Wabash Rivers," states that his "practice largely lies in the 'bottoms' of these rivers, and among farmers more or less exposed to the vicissitudes of a country that is being opened up and brought under cultivation." From May, 1875, to May, 1886, he had under observation 238 cases of croupous pneumonia, not malarial, "few of which presented the sthenic type. Under treatment with quinin, supplemented by zinc phosphide, and by camphor and digitalis, when necessary, the mortality was 6.5 per cent."

He explains that he means by typhoid pneumonia, "a croupous pneumonia that in an enfeebled constitution runs an adynamic course," and he makes the following acute comment:

"How quinia accomplishes good results . . . has not as yet been deterwished to determine, if possible, (1) how quinin exerts its favorable influence on the acute pneumonias of man, and (2) how this influence, as a whole or in any of its various phases, may be reinforced or made more definite.

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[10] Med. News, 1892, 61, p. 216. Pennsylvania Med. Jour., 1906, 9, p. 426. Internat. Clin., 1912, 3, p. 56. Jour. Am. Med. Assn., 1913, 61, p. 107.

The first of these questions leads to an investigation (1) of the germicidal powers of the quinin compounds and other cinchona derivatives; (2) of their power to modify in any way the aggressive action of the infective agent; (3) of their power to modify in any way the results of infective action, including the development and effects of any or all of the poisons taking part in the toxic complex, and (4) of their effects, direct and indirect, by way of excitation or by way of supplementation, of the various defensive processes (leukocytosis, phago-

mined. I opine that it is not by its fever-lowering property, for I have seen the general condition . . . improved even when no impression was made on the temperature. . . . Our typhoid pneumonia is very *fatal* and quinia given in large—antipyretic—doses may not reduce the morning temperature at all, and yet . . . influence for good the course of the disease. There seems to be inherent in quinia, when given in large doses, a specific power of so influencing diseases attended with great and rapid tissue metamorphosis and retention of the waste-products of such tissue changes in the system, that these diseases take on a more favorable course and come to a more happy issue under its use."

F. P. Henry, in discussing the report made by one of us in 1911 to the Association of American Physicians stated that, following Aufrecht and Petzold, he had resorted to the subcutaneous use of quinin salts continuously in his cases of pneumonia since early in 1902. "I have never published a report of my cases in detail," he added, "but in a paper . . . in the *Philadelphia Medical Journal,* February, 1903, I referred to this method of treatment, mentioned my employment of it, and heartily endorsed it. I corroborate all that Dr. Cohen says in its favor. I have not used the large doses which he employed. . . . They did not seem to me to be necessary, and this may be because the preparation which I employ . . . the hydrochlorosulphate . . . is richer in quinin than the double salt of quinin and urea."

G. A. Gibson of Edinburgh writes: "In cases showing severe toxemia and great leukocytosis, much may be done by the employment of quinin. In several instances of this kind—in which the patients had even reached a stage of profound coma, with complete relaxation of the sphincters, and every evidence of imminent danger—the hypodermic use of quinin has produced the most remarkable effect. The best preparation for this purpose is the acid hydrochloride, which is extremely soluble, and which may be administered hypodermically in doses of 2 grains every two hours, or every hour. No more gratifying results have ever occurred in my own hands than from this method of treatment."

And a few months before his death, in a letter to one of us, he wrote that by the latter's reports he had been encouraged to use quinin more boldly, especially when it was guarded by cocain or a posterior-pituitary preparation as recommended, and that he believed that he had saved the life of "a dear friend 80 years old" in that way.

cytosis, neutralization of toxic products, and the like), and specifically among these the conversion of termination from crisis into lysis; this involving, in turn, an investigation into the causes and mechanisms of natural crisis. It requires also (5) an investigation of the toxic properties of the drugs studied.

The second of these questions leads into the field which Morgenroth[11] has begun to explore; namely, chemical modification of the drug molecule. Morgenroth's drug, optochin (ethylhydrocuprein), a derivative of hydroquinin* (methylhydrocuprein), has been shown by him and others to be immeasurably superior as a pneumococcide in vitro and in vivo to any quinin compound. This work constitutes the first successful attempt toward evolving a chemotherapeutic agent for a bacterial infection, and as such, should be emphasized. However, as will appear in the course of our various reports, optochin does not exhibit the same high degree of superiority throughout the whole range of actions studied, as it does when considered from the viewpoint of bactericidal activity alone; nor do clinical reports of its use in the acute pneumonias of man show it to be greatly, if at all, superior to the ordinary salts of quinin, even when reinforced by antipneumococcic serum.† Moreover, its greater toxicity renders it less available than the more commonly used cinchonics, as a therapeutic agent in the hands of the general practitioner under the common conditions of practice. Hence, since the therapeutic problem in pneumonia is only in part one of a bactericide, and the other properties of quinin are therefore to be studied and if possible, reinforced — with a lowering rather than an elevation of toxicity — there is still much to engage the attention of workers in this special field.

This report is confined to certain preliminary studies concerning the germicidal action of quinin, its compounds, and its congeners, especially their action on pneumococci. Altho a like work had been done, in part, by previous observers, yet since the research contemplated also certain problems not as yet touched on by others, it was deemed best, both for completeness of record and to ensure consistency of methods and comparability of results throughout, to begin independently and on our own lines. In so far as we confirm previous observations, that is worth while. In so far as we differ in results from our predecessors, the question of the cause of this difference is opened.

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* Hydroquinin is found in the cinchona bark, but the commercial preparation is made synthetically. Optochin is synthetic only (see Paper 3 of this series).

† Moore[11] has shown that in experimental pneumococcal infection the conjoined use of an appropriate immune serum (according to the type of infection) and ethylhydrocuprein, affords a much greater degree of protection than the use of either alone. Lenne,[12] however, whose clinical work was based on experimental observations by Engwer and by Boehnke similar to those of Moore, did not get equally favorable results from the conjoined treatment. Using the polyvalent serum of Neufeld-Handel, he found the mortality in small groups of cases to be 11.8% with ethylhydrocuprein only; 16.5% with ethylhydrocuprein plus serum; 33% with serum only; and 30% in cases treated expectantly. These statistics are not quoted by us in proof of anything, but simply to illustrate the clinical status of the question. With ethylhydrocuprein as with quinin, both time and dose are important elements, and the sufficient dose of quinin is less dangerous than the sufficient dose of optochin.

[11] Jour. Exper. Med., 1915, 22, p. 389.

[12] Berlin. klin. Wchnschr., 1913, 50, p. 1984.

We have had of course the benefit of Morgenroth's addition to the list of chemical pneumococcides available; and part of our task has been to make various comparisons between this substance and substances derived more directly from the cinchona bark. Besides optochin and its chemical precursor hydroquinin (methylhydrocuprein) and their respective hydrochlorids, the chemicals chosen for study were the alkaloid quinin and its monobasic and dibasic salts with hydrochloric, hydrobromic, and sulfuric acids; its salicylate, lactate, arsenate, valerianate, and tannate; and the double salt of hydrochloric acid with quinin and urea. This has seemed to provide a sufficiently wide variety to permit the development of all important factors of modification. (For most of the quinin salts we are indebted to Messrs. Rosengarten and Co., who generously contributed these for experimental purposes.)

For comparison and cross tests, arsenobenzol, phenol, and mercuric chlorid were used.

Also, urea hydrochlorid, hydrochloric acid, sodium salicylate, and salicylic acid were studied by the same methods, as part of a more extended investigation into the relative influence of acid radical, basic radical, and the molecule as a whole.

Types I, II, and III of the pneumococcus were selected as the microorganisms for investigation; and for comparison and cross-testing, B. typhosus and S. aureus. (We are indebted to Dr. Rufus Cole for several of the strains of pneumococci used in these studies.)

After it had been found that except for the slightly greater resistance of Type III, there was scarcely as much difference in the results regularly obtained with the different type cultures, as at times with different individual cultures of any one type, certain observations were made with one type only (usually I or II). So, too, some tests (or tabulated reports of results) were confined to a few of the more important chemicals, after it had been found that for the special purpose in view, other differences were relatively unimportant.

In every instance, however, a sufficient number of observations, and in some tests, a multitudinous number, were made. The technic of the various tests employed is described briefly. For more detailed descriptions and discussion we refer to the second paper of this series.[13]

Morgenroth and Levy[14] found that ethylhydrocuprein possessed considerable protective power against experimental pneumococcal infections in mice and a certain degree of curative action on such infections when established. Wright and his associates[15] found that a dilution of ethylhydrocuprein 1:400,000 in serum killed pneumococci, while a dilution of 1:800,000 inhibited their growth. Practically the same results were observed in a menstruum of water. Wright used a pipet method, and his figures are therefore unusually high, the number of cocci being rather small, that is, only those of a 24-hour broth culture which adhered to a small portion of the wall of a capillary pipet. Lysol, creosote, and guaiacol tested in the same way, killed pneumococci in serum in dilutions of from 1:500 to 1:12,500; while in a menstruum of water these substances were active in dilutions ranging from 1:62,500 to 1:500,000. Tugendreich and Russo[16] found optochin in dilutions in water up to 1:16,000 bactericidal for pneumococci after an exposure of 3 hours at room temperature. Moore[17] found that ethylhydrocuprein hydrochlorid in very high dilution, in vitro, inhibited the growth of pneumococci and in lower dilutions, killed them. No constant or considerable differences were seen in these effects on typical representatives of the four groups of pneumococci. Quinin hydrochlorid was found more bactericidal for other microorganisms than for pneumococci, but proved less powerful in this respect than optochin. (Our results lead to a different conclusion as regards B. typhosus and S. aureus.) More recent observations regarding the bactericidal action of quinin in general are reported by Taylor,[18] who found quinin hydrochlorid 10 times more effective than phenol against B. aerogenes-capsulatus, and who accordingly recommends the use of this compound in the treatment of infected wounds.

In the studies here reported, we had in view (1) to determine by various methods the degree of activity and relative value of different cinchona derivatives as pneumococcidal agents in vitro; (2) to study the specificity of the action of quinin compounds and the other cinchona derivatives on pneumococci in vitro by cross-bactericidal tests with other disinfectants and other microorganisms; (3) to study the influence of acid radicals and other components of the molecules as well as of the quinin base; and (4) to study the influence of solubility.

RESULTS WITH THE CENTRIFUGATION METHOD

With this method large numbers of pneumococci were exposed to the action of the bactericide for an hour at 37 C., after which the cocci were removed, washed once by centrifugation, and then cultured. Only those compounds could be used which gave a clear solution, the less soluble salts being unsuitable for observation by this method because of the impossibility of separating the cocci from the sediment of undissolved drug. Since this test makes use (1) of large numbers of microorganisms, (2) of a high concentration of the drug, and (3) of a short time exposure, it may be regarded as germicidal in the strict sense.

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[13] Kolmer, Cohen, and Heist, Jour. Infect. Dis., 1917, 20, p. 61.

[14] Berl. klin. Wchnschr., 1911, 48, pp. 1561, 1650, 1779, 1983.

[15] Lancet, 1912, 11, pp. 1633, 1701.

[16] Ztschr. f. Immunitätsf., 1913, 19, p. 165.

[17] Jour. Exper. Med., 1915, 22, p. 269.

[18] Lancet, 1905, 11, p. 538.

The results of a number of these tests are summarized in Table 1.

TABLE 1

Results of Bactericidal Tests by the Centrifugation Method

Substance	Dilution Killing in 1 Hour		
	Type I	Type II	Type III
Ethylhydrocuprein hydrochlorid	1:800	1:700	1:700
Quinin and urea hydrochlorid	1:100	1:200	1:150
Hydroquinin hydrochlorid	1:100	1:100	1:80
Quinin bisulfate	1:100	1:80	1:80
Quinin dihydrochlorid	1:100	1:120	1:100
Quinin hydrobromid	1:100	1:100	1:100

The various salts of quinin proved bactericidal for all types in dilution of about 1:100 (least active 1:80; most active 1:200). Methylhydrocuprein (hydroquinin) hydrochlorid had about the same germicidal value as the quinin compounds.

The compound of quinin and urea hydrochlorid was slightly more active than the other quinin compounds against Type II (1:200) and Type III (1:150).

Ethylhydrocuprein hydrochlorid, however, proved 3.5 to 8 times as active as the most active of the quinin salts (bactericidal in dilution of 1:700 and 1:800).

The results with cultures of Types I, II, and III were in general approximately the same. Type III (Pneumococcus mucosus), however, in several instances proved somewhat more difficult to kill.

RESULTS WITH A PIPET METHOD

According to Wright and his associates the bactericidal power of ethylhydrocuprein hydrochlorid remains almost the same in a menstruum of serum as in salt solution. From the viewpoint of bactericidal chemotherapy this fact is important, since as Wright points out, a substance which is actively germicidal in serum may be presumed to have but slight tendency for an inactivating union with the protein of the body fluids.

With the pipet method, which permits this comparison to be made readily, we tested at 37 C., a number of compounds in a menstruum of sterile serum and in a menstruum of salt solution, using a culture of pneumococcus belonging to Type I. Altho we did not confirm Wright's observation as to retention by optochin of an activity in serum approximately equal to that in salt solution, we found its bactericidal power in serum to be high.

The results of these tests are summarized in Table 2.

TABLE 2

RESULTS OF COMPARATIVE BACTERICIDAL TESTS IN SERUM AND SALT SOLUTION BY THE PIPET METHOD

Substance	Dilution Killing in 30 Minutes	
	In Serum	In Salt Solution
Ethylhydrocuprein	1:20,000	1:100,000
Quinin and urea hydrochlorid	1:200	1:250
Hydroquinin hydrochlorid	1:500	1:750
Quinin bisulfate	1:300	1:500
Quinin hydrochlorid	1:100	1:500
Quinin tannate	1:30	1:50
Quinin dihydrobromid	1:1000	1:500
Quinin salicylate	1:300	1:500
Phenol	1:150	1:300
Mercuric chlorid	1:1000	1:10,000

While ethylhydrocuprein hydrochlorid is still highly active in a menstruum of serum, its bactericidal power is reduced to one-fifth part of that shown in normal salt solution.

The pneumococcidal power of most of the quinin salts is likewise lessened in serum, as compared with that in salt solution, but to nothing like the same degree as that of ethylhydrocuprein; so that the discrepancy in their general bactericidal power — while it appears to be much greater by this method than by any other of those used in this series of studies—is much less apparent in serum than in salt solution. For example, the ratio of germicide activity between quinin and urea hydrochlorid and ethylhydrocuprein is 250: 100,000 in salt solution (i. e., 1: 400), while in serum it is 200: 20,000 (i. e., 1: 100). In other words, the reduction in the power of optochin is 4 times that of the carbamide compound.

Surprisingly, one salt (the dihydrobromid) proved twice as bactericidal in serum as in salt solution, showing one-half the activity of optochin in the former, as against one-two-hundredth in the latter. This exceptional result suggests further study in several directions.

The pneumococcidal activity of phenol was reduced in serum to one-half, and that of mercuric chlorid to one-tenth of the power shown in salt solution.

The high bactericidal power of ethylhydrocuprein for pneumococci is evident in these tests, but its superiority and that of the quinin compounds in this connection over phenol and mercuric chlorid are far more striking in the antiseptic test, in which the drugs in low concentration are permitted to act on the pneumococci over a longer period.

RESULTS WITH THE COMBINED IN-VITRO-VIVO METHOD

In this method large numbers of highly virulent pneumococci belonging to Type I were exposed to high concentrations of ethylhydrocuprein and other cinchona derivatives at 37 C. in a menstruum of normal salt solution, and the degree of bactericidal activity of these cinchona cultures was tested in white mice by intraperitoneal injection of small amounts at different intervals up to 1 hour. It yielded fairly constant and decisive results, which are summarized in Tables 3 and 4.

TABLE 3

RESULTS OF BACTERICIDAL TESTS BY THE COMBINED IN-VITRO-VIVO METHOD *

Substance	Dilution	Exposure in Minutes				
		5	15	30	45	60
Ethylhydrocuprein hydrochlorid..	1:1000	—	—	—	—	—
	1:2000	+	—	—	—	—
	1:10000	+	+	+	+	+
Quinin and urea hydrochlorid.....	1:100	—	—	—	—	—
	1:500	+	+	—	—	—
	1:1000	+	+	+	+	+
Hydroquinin hydrochlorid.........	1:100	—	—	—	—	—
	1:500	+	+	+	—	—
	1:1000	+	+	+	+	—
Quinin bisulfate....................	1:200	+	—	—	—	—
	1:1000	+	+	+	+	+
Quinin hydrochlorid...............	1:100	—	—	—	—	—
	1:500	—	—	—	—	—
	1:1000	+	—	—	—	—
Quinin lactate......................	1:200	—	—	—	—	—
	1:500	+	+	—	—	—
	1:1000	+	+	+	+	+
Quinin salicylate..................	1:100	—	—	—	—	—
	1:500	+	—	—	—	—
	1:1000	+	+	+	+	—
Quinin arsenate....................	1:200	—	—	—	—	—
	1:500	+	+	—	—	—
	1:1000	+	+	+	+	+
Quinin (alkaloid)..................	1:100	—	—	—	—	—
	1:200	—	—	—	—	—
	1:500	+	+	—	—	—
	1:1000	+	+	+	+	+
Controls...........................	....	+	+	+	+	+

* Pneumococcus Type I used, of which the M. L. D. for mice was 0.000001 c.c. of a 24-hour broth culture.
+ = animal died. — = animal living (48 hours).

TABLE 4

RESULTS OF BACTERICIDAL TESTS BY THE COMBINED IN-VITRO-VIVO METHOD (PNEUMOCOCCUS TYPE I)

Substance	Dilution	Exposure in Minutes				
		5	15	30	45	60
Phenol	1:100	—	—	—	—	—
	1:200	+	+	+	—	—
	1:500	+	+	+	+	+
Bichlorid of mercury	1:100	—	—	—	—	—
	1:200	+	+	—	—	—
	1:500	+	+	+	+	—
Controls		+	+	+	+	+

+ = animal died. — = animal living (48 hours).

In certain instances the quinin salts were used in concentrations greater than their maxima of solubility in salt solution, but the presence of undissolved particles did not appear to interfere with the regularity of the results.

Altho the mice used in these experiments were kept under observation for 6 days, the tables were prepared according to the results observed within 48 hours. After this time the results were irregular; some of the animals, as shown by necropsy (see third paper of this series), succumbing to the toxic effects of the drugs rather than to an infection with pneumococci.

Ethylhydrocuprein hydrochlorid proved 10 times more bactericidal in the 5-minute period of exposure, and from 5 to 10 times more active in the 15-minute period, than the majority of the quinin compounds, phenol, and mercuric chlorid. Exposure of the culture (Type I) to a solution of 1:2000 for 15 minutes, or of 1:1000 for 5 minutes, was sufficient to prevent infection of the animals.

Quinin hydrochlorid, however, in both the 5-minute and 15-minute periods, was half as active as optochin; the alkaloid quinin and quinin arsenate one-fifth as active.

After an exposure of 30 minutes, and still more after one of 60 minutes, the difference between the bactericidal activity of ethylhydrocuprein and that of the quinin compounds in general was markedly lessened; the former being only 2 to 4 times more active than the latter. The compound of quinin and urea hydrochlorid was germicidal in dilution of 1:500 after 30 minutes' exposure, and quinin salicylate in 1:500 after 15 minutes' exposure.

TABLE 5

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (PNEUMOCOCCUS TYPE I)

Salts	Percentage of Alkaloid	Dilutions							
		1:1000	1:2000	1:4000	1:5000	1:8000	1:10,000	1:16,000	1:20,000
Quinin tannate	20.0	—	—	—	—	—	—	—	—
Quinin and urea hydrochlorid	58.0	—	—	—	—	—	—	+	+
Quinin bisulfate	59.1	—	—	—	—	—	—	—	—
Quinin dihydrobromid	60.0	—	—	—	—	—	—	—	—
Quinin arsenate	69.4	—	—	—	—	—	—	—	—
Quinin salicylate	70.1	—	—	—	—	—	—	—	—
Quinin dihydrochlorid	72.0	—	—	—	—	—	—	—	—
Quinin valerinate	73.0	—	—	—	—	—	—	—	—
Quinin sulfate	73.5	—	—	—	—	—	—	—	—
Quinin hydrobromid	76.0	—	—	—	—	—	—	—	—
Quinin phosphate	76.2	—	—	—	—	—	—	—	—
Quinin lactate	78.2	—	—	—	—	—	—	—	—
Quinin hydrochlorid	81.8	—	—	—	—	—	—	—	—
Quinin alkaloid	100.0	—	—	—	—	—	—	—	—
Ethylhydrocuprein hydrochlorid	75-80	—	—	—	—	—	—	—	—
Hydroquinin hydrochlorid	90.8	—	—	—	—	—	—	—	—

+ = growth. — = sterility.

TABLE 6

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (PNEUMOCOCCUS TYPE II)

Salts	Percentage of Alkaloid	Dilutions							
		1:1000	1:2000	1:4000	1:5000	1:8000	1:10,000	1:16,000	1:20,000
Quinin tannate	20.0	—	—	—	—	—	—	—	—
Quinin and urea hydrochlorid	58.0	—	—	—	—	—	—	—	—
Quinin bisulfate	59.1	—	—	—	—	—	—	—	—
Quinin dihydrobromid	60.0	—	—	—	—	—	—	—	—
Quinin arsenate	69.4	—	—	—	—	—	—	—	—
Quinin salicylate	70.1	—	—	—	—	—	—	—	—
Quinin dihydrochlorid	72.0	—	—	—	—	—	—	—	—
Quinin valerinate	73.0	—	—	—	—	—	—	—	—
Quinin sulfate	73.5	—	—	—	—	—	—	—	—
Quinin hydrobromid	76.0	—	—	—	—	—	—	—	—
Quinin phosphate	76.2	—	—	—	—	—	—	—	—
Quinin lactate	78.2	—	—	—	—	—	—	—	—
Quinin hydrochlorid	81.8	—	—	—	—	—	—	—	—
Quinin alkaloid	100.0	—	—	—	—	—	—	—	—
Ethylhydrocuprein hydrochlorid	75-80	—	—	—	—	—	—	—	—
Hydroquinin hydrochlorid	90.8	—	—	—	—	—	—	—	—

+ = growth. — = sterility.

TABLE 7

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (PNEUMOCOCCUS TYPE III)

Salts	Percentage of Alkaloid	Dilutions							
		1:666	1:1000	1:2000	1:4000	1:5000	1:8000	1:10,000	1:16,000
Quinin tannate	20.0	—	—	—	—	—	+	+	+
Quinin and urea hydrochlorid	58.0	—	—	—	—	—	—	—	—
Quinin bisulfate	59.1	—	—	—	—	—	—	—	—
Quinin dihydrobromid	60.0	—	—	—	—	—	—	—	—
Quinin arsenate	69.4	—	—	—	—	—	—	—	—
Quinin salicylate	70.1	—	—	—	—	—	—	—	—
Quinin dihydrochlorid	72.0	—	—	—	—	—	—	—	—
Quinin valerinate	73.0	—	—	—	—	—	—	—	—
Quinin sulfate	73.5	—	—	—	—	—	—	—	—
Quinin hydrobromid	76.0	—	—	—	—	—	—	—	—
Quinin phosphate	76.2	—	—	—	—	—	—	—	—
Quinin lactate	78.2	—	—	—	—	—	—	—	—
Quinin hydrochlorid	81.8	—	—	—	—	—	—	—	—
Quinin alkaloid	100.0	—	—	—	—	—	—	—	—
Ethylhydrocuprein tydrochlorid	75-80	—	—	—	—	—	—	—	—
Hydroquinin hydrochlorid	90.8	—	—	—	—	—	—	—	—

+ = growth. — = sterility.

TABLE 5.—(*Continued*)

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (PNEUMOCOCCUS TYPE I)

Dilutions									
1:50,000	1:80,000	1:100,000	1:200,000	1:800,000	1:1,000,000	1:1,500,000	1:2,000,000	1:3,000,000	1:4,000,000
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
—	—	+	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
—	+	+	+	+	+	+	+	+	+
—	+	+	+	+	+	+	+	+	+
—	+	+	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
—	—	—	—	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
—	—	—	—	—	—	+	+	+	+
—	—	+	+	+	+	+	+	+	+

TABLE 6—(*Continued*)

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (PNEUMOCOCCUS TYPE II)

Dilutions									
1:50,000	1:80,000	1:100,000	1:200,000	1:800,000	1:1,000,000	1:1,500,000	1:2,000,000	1:3,000,000	1:4,000,000
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
—	—	+	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
—	—	—	—	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
—	—	+	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
—	—	+	+	+	+	+	+	+	+
—	—	—	+	+	+	+	+	+	+
—	—	—	—	+	+	+	+	+	+
—	+	+	+	+	+	+	+	+	+
—	—	—	—	—	—	—	—	+	+
—	+	+	+	+	+	+	+	+	+

TABLE 7.—(*Continued*)

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (PNEUMOCOCCUS TYPE III)

Dilutions									
1:20,000	1:50,000	1:80,000	1:100,000	1:200,000	1:800,000	1:1,000,000	1:1,500,000	1:2,000,000	1:4,000,000
+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
—	—	—	—	+	+	+	+	+	+
—	+	+	+	+	+	+	+	+	+
—	—	—	—	+	+	+	+	+	+
—	—	—	—	+	+	+	+	+	+
—	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+
—	+	+	+	+	+	+	+	+	+
—	—	—	—	+	+	+	+	+	+
—	+	+	+	+	+	+	+	+	+
—	+	+	+	+	+	+	+	+	+
—	—	—	—	+	+	+	+	+	+
—	+	+	+	+	+	+	+	+	+
—	—	—	—	—	—	—	—	+	+
—	+	+	+	+	+	+	+	+	+

RESULTS WITH THE ANTISEPTIC METHOD

In the method which we have termed 'antiseptic' small numbers of the microorganisms are exposed to high dilutions of the disinfectant over a long period of time. This form of test has brought out more clearly the efficacy and high degree of specificity of cinchona derivatives as pneumococcides.

A large number of such experiments have been made with a number of quinin compounds, with optochin, and with hydroquinin, on cultures of pneumococci belonging to Types I, II, and III, the results being summarized in Tables 5, 6, and 7.

All these tests were conducted with solutions of the drugs in broth and at a temperature of 37 C. The results tabulated were noted after an 8-day period of observation. Had the readings been recorded as observed at the end of the first 24 hours, the values shown would have been much higher, since many tubes of the lower dilutions were apparently sterile at this time, which later evidenced the growth of pneumococci. No tabulation of this retarding effect is here presented, but a record of the fact is thus made.

Ethylhydrocuprein hydrochlorid inhibits the multiplication of pneumococci belonging to the first three serologic types, in dilutions varying from 1: 1,000,000 to 1: 2,000,000 (Tables 5, 6, and 7).

The results with the quinin salts differ considerably from one another, the inhibiting dilutions ranging from 1: 5000 and 1: 20,000 (quinin tannate) to 1: 100,000 and 1: 200,000 (quinin hydrobromid), or from one-two-hundredth to one-tenth the power of ethylhydrocuprein. Quinin alkaloid ranges from 1: 20,000 against Type III to 1: 50,000 against Type II.

According to the calculated amounts of quinin contained in the various salts tested (see Tables 5, 6, and 7) there is no definite relation apparent between the percentage of alkaloid present and the antiseptic power of the compound. Tests to determine the antiseptic value of the various acid radicals and other suggested factors of modification are under way, but are not sufficiently numerous, as yet, to warrant conclusions.

The quinin and urea hydrochlorid compound was relatively low in antiseptic value in these tests (1: 10,000 for Type I, 1: 20,000 for Type II, 1: 16,000 for Type III), altho with other methods, or germicidal tests as we have called them, in which low dilutions were used with short exposure — as also with the plating method, later described, which gives both antiseptic and germicidal values — this double salt yielded results which compared favorably with, and in some instances were much better than, those with other quinin compounds. (This tends to confirm the view that the bactericidal effect is neither the only nor the chief value of quinin compounds in general, and of the carbamide in particular, as therapeutic agents in pneumonia.) Urea hydrochlorid was found without demonstrable bactericidal properties over pneumococci in dilutions as low as 1: 100 (Table 8), tho Symmers and Kirk[19] found that urea was active as a germicide in very low dilutions and advocated it in the treatment of infected wounds. In so far as the double hydrochlorid of quinin and urea is concerned, the bactericidal activity on pneumococci is to be ascribed to the quinin salt.

TABLE 8

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (EFFECT OF UREA HYDROCHLORID ON PNEUMOCOCCUS TYPE I)

No.	Culture	Broth	Dilutions	Results (Days)							
				1	2	3	4	5	6	7	8
1	0.05 c.c.	10 c.c.	1:100	+	+	+	+	+	+	+	+
2			1:150	+	+	+	+	+	+	+	+
3			1:200	+	+	+	+	+	+	+	+
4			1:300	+	+	+	+	+	+	+	+
5			1:500	+	+	+	+	+	+	+	+
6			1:1000	+	+	+	+	+	+	+	+
7			Control	+	+	+	+	+	+	+	+

+ = growth.

TABLE 9

SUMMARY OF RESULTS OF BACTERICIDAL TESTS ON DIFFERENT TYPES OF PNEUMOCOCCI BY THE ANTISEPTIC METHOD

Substance	Dilutions in Which the Various Pneumococci Were Killed		
	Type I	Type II	Type III
Ethylhydrocuprein hydrochlorid	1:1,000,000	1:2,000,000	1:1,500,000
Hydroquinin hydrochlorid	1: 80,000	1: 50,000	1: 20,000
Quinin and urea hydrochlorid	1: 10,000	1: 20,000	1: 16,000
Quinin (alkaloid)	1: 20,000	1: 50,000	1: 20,000
Quinin bisulfate	1: 80,000	1: 80,000	1: 100,000
Quinin tannate	1: 20,000	1: 20,000	1: 5,000
Quinin dihydrobromid	1: 100,000	1: 100,000	1: 20,000
Quinin arsenate	1: 100,000	1: 200,000	1: 100,000
Quinin salicylate	1: 50,000	1: 100,000	1: 100,000
Quinin dihydrochlorid	1: 50,000	1: 20,000	1: 20,000
Quinin valerinate	1: 50,000	1: 80,000	1: 16,000
Quinin sulfate	1: 200,000	1: 100,000	1: 20,000
Quinin hydrobromid	1: 200,000	1: 100,000	1: 20,000
Quinin phosphate	1: 100,000	1: 80,000	1: 20,000
Quinin lactate	1: 100,000	1: 100,000	1: 20,000
Quinin hydrochlorid	1: 100,000	1: 200,000	1: 100,000

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[19] Lancet, 1915, 11, p. 1237.

The inhibiting influence of the cinchona derivatives was about the same on Types I and II of pneumococci. With Type III somewhat lower values were found; that is, this type was apparently less susceptible to the action of quinin (Table 9). Because, however, of the irregularities exhibited by different cultures belonging to the same type we are not inclined to lay too much stress on these differences (see Table 14).

RESULTS WITH A PLATING METHOD

A summary of the results observed with a plating method employing ethylhydrocuprein hydrochlorid and 4 other cinchona derivatives and a culture of pneumococcus belonging to Type I, is shown in Table 10.

The bactericide in varying dilutions was allowed to remain in contact with the cocci for a period of 24 hours at 37 C. Then subcultures were made in 10 c.c. of blood agar.

TABLE 10

Summary of Results of Bactericidal Tests by the Plating Method (the Bactericidal Action of Cinchona Derivatives on Pneumococcus Type I)

Substance	Highest Dilution Causing	
	Inhibition	Sterility
Ethylhydrocuprein hydrochlorid	1:1,000,000	1:40,000
Hydroquinin hydrochlorid	1: 60,000	1: 7000
Quinin and urea hydrochlorid	1: 60,000	1: 6000
Quinin bisulfate	1: 40,000	1: 5000
Quinin hydrochlorid	1: 40,000	1: 4000
Urea hydrochlorid	Not in 1:100	Not in 1:100

The particular advantage of this method lies in its showing under identical conditions both the inhibiting, or antiseptic, and the killing, or bactericidal, dose of the chemical studied.

The inhibiting dose of the various compounds (exposure 24 hours) was from 8 to 25 times less than the bactericidal dose, ethylhydrocuprein proving by far the most active (1:1,000,000 and 1:40,000), and hydroquinin and quinin and urea hydrochlorid next in both inhibiting (1:60,000) and sterilizing power (1:7000 and 1:6000) in all these tests (Table 10).

INFLUENCE OF PERIOD OF EXPOSURE

The evidence at hand indicates that quinin and its derivatives (under which term we may broadly include optochin as well as hydroquinin) act somewhat slowly on microorganisms and demonstrate more clearly their specificity for the pneumococcus in the antiseptic tests (with long exposures) than in the germicidal tests (in which the exposure is brief). This fact is further illustrated by the results presented in Tables 2, 4, and 13. In all of the tests there set forth, phenol and mercuric chlorid were used as standards of comparison; and were shown to rank fairly high as germicides for the pneumococcus (Tables 2 and 4), but to fall far below the quinin derivatives in the antiseptic tests (Table 13).

THE SPECIFICITY OF THE BACTERICIDAL ACTION OF ETHYLHYDROCUPREIN HYDROCHLORID AND OTHER CINCHONA (QUININ) DERIVATIVES ON PNEUMOCOCCI IN VITRO

The specificity of the bactericidal action of cinchona derivatives on pneumococci was studied by cross-testing their efficacy against B. typhosus and S. aureus; while also testing the bactericidal activity of phenol, mercuric chlorid, arsenobenzol, and other chemicals on pneumococci.

In a general way the results of these tests show conclusively that, in long-exposure tests at least, the quinin derivatives possess a high degree of specific pneumococcidal activity.

The results of tests with various quinin compounds and optochin, using the technic of the antiseptic method, and B. typhosus and S. aureus as the test microorganisms, are shown in Tables 11 and 12.

In a general way the results observed with both microorganisms and all the quinin salts were similar; that is, the inhibiting dilution usually ranged from 1:1000 to 1:2000. Ethylhydrocuprein hydrochlorid was slightly the more bactericidal for B. typhosus (1:2500), and decidedly the more so for S. aureus (1:5000) (Tables 11 and 12)

The value of ethylhydrocuprein as a bactericide was from 200 to 400 times greater for the pneumococcus than for B. typhosus and the staphylococcus; the various quinin derivatives showed a bactericidal activity against the pneumococcus ranging from 5 to 50 times greater than against the other microorganisms (Table 13).

Arsenobenzol in dilution of 1:2500 was effective against pneumococci, and in dilutions of from 1:1000 to 1:2000, against B. typhosus and staphylococci, but far inferior to the quinin derivatives as a pneumococcide. Likewise phenol, mercuric chlorid, and sodium salicylate proved far less active than the cinchona derivatives as pneumococcides.

TABLE 11

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (THE BACTERICIDAL ACTION OF QUININ SALTS AND DERIVATIVES ON B. TYPHOSUS)

Substance	Percentage of Alkaloid	Dilutions						
		1:100	1:200	1:300	1:400	1:500	1:600	1:700
Quinin tannate	20.0	—	—	—	—	—	—	—
Quinin and urea hydrochlorid	58.0	—	—	—	—	—	—	—
Quinin bisulfate	59.1	—	—	—	—	—	—	—
Quinin dihydrobromid	60.0	—	—	—	—	—	—	—
Quinin arsenate	69.4	—	—	—	—	—	—	—
Quinin salicylate	70.1	—	—	—	—	—	—	—
Quinin dihydrochlorid	72.0	—	—	—	—	—	—	—
Quinin valerinate	73.0	—	—	—	—	—	—	—
Quinin sulfate	73.5	—	—	—	—	—	—	—
Quinin hydrobromid	76.6	—	—	—	—	—	—	—
Quinin phosphate	76.2	—	—	—	—	—	—	—
Quinin lactate	78.2	—	—	—	—	—	—	—
Quinin hydrochlorid	81.8	—	—	—	—	—	—	—
Quinin alkaloid	100.0	—	—	—	—	—	—	—
Ethylhydrocuprein hydrochlorid	90.8	—	—	—	—	—	—	—
Hydroquinin hydrochlorid	75-80	—	—	—	—	—	—	—
Arsenobenzol	....	—	—	—	—	—	—	—

+ = growth. — = sterility.

TABLE 12

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (THE BACTERICIDAL ACTION OF QUININ SALTS AND DERIVATIVES ON STAPHYLOCOCCUS AUREUS)

Substance	Percentage of Alkaloid	Dilutions						
		1:100	1:200	1:300	1:400	1:500	1:600	1:700
Quinin tannate	20.0	—	—	—	—	—	—	—
Quinin and urea hydrochlorid	58.0	—	—	—	—	—	—	—
Quinin bisulfate	59.1	—	—	—	—	—	—	—
Quinin dihydrobromid	60.0	—	—	—	—	—	—	—
Quinin arsenate	69.4	—	—	—	—	—	—	—
Quinin salicylate	70.1	—	—	—	—	—	—	—
Quinin dihydrochlorid	72.0	—	—	—	—	—	—	—
Quinin valerinate	73.0	—	—	—	—	—	—	—
Quinin sulfate	73.5	—	—	—	—	—	—	—
Quinin hydrobromid	76.6	—	—	—	—	—	—	—
Quinin phosphate	76.2	—	—	—	—	—	—	—
Quinin lactate	78.2	—	—	—	—	—	—	—
Quinin hydrochlorid	81.8	—	—	—	—	—	—	—
Quinin alkaloid	100.0	—	—	—	—	—	—	—
Ethylhydrocuprein hydrochlorid	75-80	—	—	—	—	—	—	—
Hydroquinin hydrochlorid	....	—	—	—	—	—	—	—
Arsenobenzol	....	—	—	—	—	—	—	—

+ = growth. — = sterility.

TABLE 11.—(*Continued*)

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (THE BACTERICIDAL ACTION OF QUININ SALTS AND DERIVATIVES ON B. TYPHOSUS)

Dilutions											
1:800	1:900	1:1000	1:2000	1:3000	1:4000	1:5000	1:6000	1:7000	1:8000	1:9000	1:10000
−	−	+	+	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	−	−	−	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+

TABLE 12.—(*Continued*)

RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (THE BACTERICIDAL ACTION OF QUININ SALTS AND DERIVATIVES ON STAPHYLOCOCCUS AUREUS)

Dilutions											
1:800	1:900	1:1000	1:2000	1:3000	1:4000	1:5000	1:6000	1:7000	1:8000	1:9000	1:10000
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+
−	−	−	−	−	−	−	+	+	+	+	+
−	−	−	−	+	+	+	+	+	+	+	+
−	−	−	+	+	+	+	+	+	+	+	+

Hydrochloric and salicylic acids proved so much less bactericidal than their respective salts with quinin that so far as these tests go, the base is evidently the active constituent.

TABLE 13

SUMMARY OF RESULTS OF BACTERICIDAL TESTS BY THE ANTISEPTIC METHOD (EFFECT OF QUININ DERIVATIVES AND OTHER DISINFECTANTS ON VARIOUS MICROORGANISMS)

Substance	Dilutions that Killed		
	Pneumococcus Type I	B. Typhosus	S. Aureus
Ethylhydrocuprein hydrochlorid	1:1,000,000	1:2500	1:5000
Quinin and urea hydrochlorid	1: 10,000	1:2000	1:2000
Hydroquinin hydrochlorid	1: 50,000	1:2000	1:2000
Quinin bisulfate	1: 80,000	1:1000	1:2000
Quinin dihydrobromid	1: 100,000	1:2000	1:1000
Quinin salicylate	1: 50,000	1:1000	1:1000
Quinin tannate	1: 20,000	1:1000	1:1000
Quinin (alkaloid)	1: 20,000	1:2000	1:1000
Arsenobenzol	1: 2500	1:2000	1:1000
Phenol	1: 1000	1: 125	1: 80
Bichlorid of mercury	1: 5000	1:20,000	1:20,000
Urea hydrochlorid	Not in 1:100	Not in 1:100	Not in 1:100
Salicylic acid	1: 1000	0*	0
Sodium salicylate	1: 300	0	0
Hydrochloric acid	1: 100	0	0

* 0 = not tested.

TABLE 14

VARIATION IN RESISTANCE TO BACTERICIDAL AGENTS AMONG CULTURES OF THE SAME TYPE OF PNEUMOCOCCUS

Substance	Method	Highest Dilution that Killed			
		Type I		Type II	
		A	B	A	B
Ethylhydrocuprein hydrochlorid	Antiseptic	1:1,000,000	1:1,500,000	1:2,000,000	1:3,200,000
	Plating	1: 40,000	1: 20,000	1: 80,000	1: 120,000
	Pipet*	1: 100,000	1: 200,000	1: 150,000	1: 120,000
Hydroquinin hydrochlorid	Antiseptic	1: 80,000	1: 60,000	1: 50,000	1: 100,000
	Plating	1: 6000	1: 14,000	1: 8000	1: 10,000
	Pipet	1: 500	1: 400	1: 600	1: 1000
Quinin and urea hydrochlorid	Antiseptic	1: 10,000	1: 40,000	1: 20,000	1: 30,000
	Plating	1: 6000	1: 8000	1: 8000	1: 8000
	Pipet	1: 200	1: 200	1: 250	1: 400

* In serum in all tests.

VARIATION IN THE BACTERICIDAL ACTION OF CINCHONA DERIVATIVES ON DIFFERENT STRAINS OF PNEUMOCOCCI BELONGING TO THE SAME TYPE

Moore found that there were practically no differences among pneumococci belonging to the four serologic types in their resistance to the bactericidal action of ethylhydrocuprein and quinin hydrochlorid. As mentioned, we have observed on occasions that Type III (Pneumococcus mucosus) was slightly more difficult to kill than Types I and II.

A number of experiments, after various methods, with different cultures belonging to the same serologic types, developed, however, considerable variations in resistance. A few of these experiments with Types I and II and 3 cinchona derivatives, are summarized in Table 14.

As shown in the table, the variations in resistance were frequently of considerable magnitude. In all our preceding tables, therefore, we have taken the lowest figures. We are unable to offer an adequate explanation of these differences, but experiments have suggested that freshly isolated strains, and strains freshly passed through mice with marked increase of virulence, are more resistant than the same strains carried for some time in artificial culture media.

CONCLUSIONS

Certain cinchona derivatives — quinin, hydroquinin (methylhydrocuprein), optochin (ethylhydrocuprein), and their salts — tested by various methods, exert a very high bactericidal power on pneumococci in vitro, ethylhydrocuprein being by far the most active. Similar studies of other cinchona alkaloids with their compounds and modifications are to be desired.

The results of bactericidal tests numerically expressed, vary greatly according to the technic employed and according to such factors as temperature, duration of exposure, and vigor and virulence of the microorganisms. Stated and standardized methods are therefore necessary, since only those results that are obtained by the same technic, are susceptible of direct comparison.

While the various cinchona derivatives studied exhibit considerable differences in relative bactericidal value, it is probable that the power of any one of them is about the same for all types of pneumococci, tho in several instances Type III (Pneumococcus mucosus) was found somewhat more resistant than the other two types studied. The fact that the different quinin compounds do not, as might have been presumed, vary in bactericidal activity according to their respective alkaloidal strengths, makes needful a further investigation of the relative influence of acid and basic radicals and of the molecule as a whole.

Different cultures of pneumococci belonging to the same serologic type exhibit varying degrees of resistance to bactericidal agents. It is probable that this variation bears a relation to virulence, and more particularly to whether the strain has been freshly isolated from lesions or has been cultivated for a prolonged period on an artificial medium.

The cinchonics studied act slowly on pneumococci, the best bactericidal and inhibiting values (that is, the most marked activity of high dilutions — from about 1:10,000 to 1:2,000,000) being shown when the number of microorganisms is small and the exposure relatively prolonged. Nevertheless, even with brief exposures (5 to 60 minutes) and large numbers of virulent microorganisms, moderately high concentrations (from 1:100 to 1:2000) are germicidal.

Cross bactericidal tests — employing, on the one hand, quinin and optochin compounds, with cultures of B. typhosus and of a staphylococcus, and on the other hand, arsenobenzol, phenol, mercuric chlorid, sodium salicylate, and other substances, with cultures of pneumococci — show that the cinchona derivatives possess a high degree of specific pneumococcidal activity.

The pneumococcidal activity of ethylhydrocuprein hydrochlorid was reduced to one-fifth or sometimes one-tenth part in a menstruum of serum, as compared with that shown in normal salt solution. The activity of the majority of the quinin salts tested was, relatively, but slightly reduced in serum. The superiority of optochin over quinin was thus, to a certain extent, lessened. The activity of phenol in serum was reduced to one-half, and that of mercuric chlorid to one-tenth, of their respective values in salt solution. For bactericidal chemotherapy it is desirable that agents to be used clinically maintain a high degree of germicidal power in serum, in tests in vitro.